

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

---

Nebraska Beef Cattle Reports

Animal Science Department

---

January 1999

## N-alkane as an Internal Market for Predicting Digestibility of Forages

Russell Sandberg

*University of Nebraska-Lincoln*

Don C. Adams

*University of Nebraska-Lincoln*, [dadams1@unl.edu](mailto:dadams1@unl.edu)

Terry J. Klopfenstein

*University of Nebraska-Lincoln*, [tklopfenstein1@unl.edu](mailto:tklopfenstein1@unl.edu)

Rick Grant

*University of Nebraska-Lincoln*

Follow this and additional works at: <https://digitalcommons.unl.edu/animalscinbcr>

 Part of the [Animal Sciences Commons](#)

---

Sandberg, Russell; Adams, Don C.; Klopfenstein, Terry J.; and Grant, Rick, "N-alkane as an Internal Market for Predicting Digestibility of Forages" (1999). *Nebraska Beef Cattle Reports*. 420.

<https://digitalcommons.unl.edu/animalscinbcr/420>

This Article is brought to you for free and open access by the Animal Science Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Nebraska Beef Cattle Reports by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

volatilization versus 46 pounds per head for CON. This decrease can be primarily attributed to the decrease in N excreted by the steers and some improvement in N captured in manure.

Dietary N and indigestibility of dietary OM, rather than ingredient composition, runoff amounts and variation in pen cleaning, may be the largest factors influencing manure characteristics, because considerable differences from year one to year two were observed in all measurements for nutrient balance except the manure data. Averaged across both treatments, N in manure for the summer trials were 16.13 and 16.39 ± .73 lb per head for years one and two, respectively. Organic matter in manure was 323 (year 1) and 315 (year 2) ± 14 pounds per head for the summer. Likewise, N in manure from the winter/spring trials were 39.6 and 45.4 ± 2.6 pounds for years one and two, respectively.

It appears decreasing dietary protein to animal requirements will decrease nitrogen excretion. With less nitrogen excreted, nitrogen losses via volatilization can be minimized. However, it appears there is an interaction between OM and N on the pen surface. If OM excretion is increased, more N will be "trapped" in the manure and may eventually be utilized as fertilizer N, improving nutrient balance by decreasing losses from the feedlot. Volatilization is more of an issue during the summer months, due to ambient temperature, but some N losses occur year-round through volatilization or runoff (what little may occur).

<sup>1</sup>Galen Erickson, graduate student, Terry Klopfenstein, professor, Todd Milton, assistant professor, Dan Herold, research technician, Animal Science, Lincoln.

<sup>2</sup>Author would like to acknowledge the tremendous help of the feedlot and lab personnel in collection and analysis of a large number of samples.

# N-alkane as an Internal Marker for Predicting Digestibility of Forages

Russell Sandberg  
Don Adams  
Terry Klopfenstein  
Rick Grant<sup>1</sup>

N-alkanes may be used as internal markers to predict forage digestibility and may be a suitable alternative to other traditionally used internal markers.

## Summary

*Independent digestion trials were conducted with three immature grasses, mature grass hay, and alfalfa hay to compare n-alkane with indigestible ADF (IADF) as internal markers to predict in vivo dry matter digestibility (DMD). Forage DMD estimated with n-alkane ratios were lower than in vivo DMD. N-alkanes predicted higher DMD than IADF for alfalfa hay and two of the immature grasses. Comparison of freeze-drying and oven-drying on fecal n-alkane concentrations showed oven-drying reduced amounts of n-alkane extracted for alfalfa hay but had no effect on grass hay. Although fecal recovery of markers was incomplete, more n-alkane was recovered than IADF.*

## Introduction

Forage dry matter digestibility (DMD) is an essential tool for assessing the nutrient status of grazing animals. While many different methods have been developed to estimate DMD, errors present due to variation in dry matter intake, physical form of the forage, and age and species of the animals can be corrected with an internal marker (indigestible plant component recoverable

in the feces). Several internal markers, such as acid insoluble ash, lignin and indigestible detergent fibers, have been investigated for their potential to estimate digestibility; however, none of these markers exhibit the characteristics of an ideal internal marker.

In recent years, it has been proposed long chain hydrocarbons (n-alkanes) found in plant cuticular wax may be utilized as internal markers. The n-alkanes found in most pasture species have odd-numbered carbon chains containing 25-35 carbon atoms. Because fecal recovery of n-alkanes improves with increasing chain length, C<sub>32</sub>:C<sub>33</sub> is usually selected. However, C<sub>32</sub> and C<sub>33</sub> are not always present in sufficient quantities to be utilized as internal markers.

The objectives of our study were to identify which n-alkane was present in sufficient quantities to be used as an internal marker, compare its effectiveness as an internal marker with indigestible acid detergent fiber (IADF) for five forages differing in maturity, compare effects of different drying methods on n-alkane extraction from feces and determine if n-alkane disappearance observed during passage through the gastrointestinal tract occurs in the rumen.

## Procedure

Five yearling steers (avg body weight = 925 lb) were housed individually in 10 ft x 10 ft pens for five independent digestion trials using mixed grasses from subirrigated meadow (meadow), meadow regrowth and native Sandhills range (range), mature mixed grass hay from meadow and alfalfa hay. The meadow, range, and meadow regrowth trials were conducted in 1995 using immature grasses, beginning June 1, July 1 and

(Continued on next page)

July 15, respectively, at the Gudmundsen Sandhills Research Laboratory located near Whitman, Nebraska. The meadow hay and alfalfa hay trials were conducted in September 1996 at the West Central Research and Extension Center, North Platte, Nebraska.

Each trial consisted of a 10-day diet adaptation period followed by five days of total fecal collection. Each forage was limit-fed twice daily at 2 percent of body weight with forage from meadow, meadow regrowth and range harvested 0.5 hours before feeding. Feed samples were collected and frozen before each feeding, refusals were collected and frozen before the morning feeding and feces were collected and frozen twice daily.

To examine  $C_{31}$  disappearance in the rumen, forages collected during digestion trials were initially subjected to *in vitro* fermentation in which samples weighing 1.8 g were measured into three *in vitro* tubes in 0.6 g increments, inoculated with a mixture of rumen fluid: McDougall's buffer, and were incubated in a 39°C water bath for either 48 or 96 hours. The contents from the three tubes were filtered through filter paper and the residues were combined to form a single sample and saved for later n-alkane analysis.

#### Laboratory Analyses

Diets, refusals and feces for each trial were freeze dried and ground to pass through a 1-mm screen using a Wiley mill. Samples were composited across days on a per animal basis. Feces from the meadow hay and alfalfa hay trials were subsampled and either freeze dried or dried in a forced air oven (60°C) to compare the effect of drying method on n-alkane extraction. Laboratory analysis included DM, organic matter and IADF. N-alkane analysis was conducted by placing either 1 g of feces or 2 g of forage in a 75-ml tube with 0.6 ml of a 1,000 ppm solution of  $C_{32}$  in n-hexane as an internal standard. Ten ml of alcoholic potassium hydroxide were added to each sample and the samples were placed in a water bath at 90°C for 4.5 hours. Upon removal from the water bath, 7 ml of n-hexane

and 2 ml of water were added to each tube. After shaking vigorously, samples were centrifuged and the n-hexane layer was transferred to a prepared column for solid phase separation. The column was prepared by first placing 2 g of silicic acid per column in a 110°C oven to activate the silicic acid. Then the silicic acid was suspended in solution using 10 ml of n-hexane and placed in an extraction column. The extract eluted from the column was evaporated, and reconstituted with 2 ml of n-hexane and placed into a glass vial for later analysis using gas chromatography. Ten samples from the meadow, meadow regrowth and range trials selected randomly were reconstituted with 2 ml of a triacontane standard (0.3 mg per ml of n-hexane) to determine the recovery rate of dotriacontane during the extraction procedure.

#### Results

N-alkane  $C_{31}$  was selected as the internal marker to estimate DMD. Although n-alkane recovery increases with increasing chain length,  $C_{33}$  was not detected in the freshly harvested forages, most likely due to a lack of column sensitivity where amounts less than 20 mg/kg are not detectable. Even though the efficiency of the procedure averaged 82 percent, this procedure uses both the natural and synthetic n-alkane ratios as a correction factor when efficiencies are less than 100 percent.

For all forages, the digestibility estimates (Table 1) calculated using n-alkane ratio were lower ( $P < .01$ ) than *in vivo* DMD. Comparison of digestibilities estimated using the n-alkane ratios and IADF ratios showed that n-alkane ratios predicted higher

DMD for meadow ( $P < .01$ ), meadow regrowth ( $P = .06$ ), and alfalfa hay ( $P = .06$ ), and lower DMD for meadow hay ( $P < .02$ ). Forage digestibilities for native range using n-alkane ratio tended to be higher ( $P = .14$ ) than IADF ratio values. Although  $C_{31}$  consistently underestimated the *in vivo* digestibilities for all forages examined, it offered an improvement over digestibilities estimated with IADF for the freshly harvested forages. *In vitro* dry matter disappearance appeared to produce estimates of digestibility comparable to the n-alkane ratio method for immature, freshly harvested forages and higher estimates for alfalfa and meadow hay.

Replacing freeze-drying of fecal samples with oven-drying would decrease the amount of drying time and increase the number of samples handled. While  $C_{31}$  amounts in feces from steers fed meadow hay were not affected ( $P > 0.10$ ) by drying method, oven-drying reduced ( $P < 0.01$ ) the amount of  $C_{31}$  recovered from the feces of steers on an alfalfa hay diet by 20 percent. During oven-drying, the high temperatures may subject  $C_{31}$  to either degradation or chemical reactions that make complete extraction difficult. Since  $C_{31}$  concentrations vary with drying methods in both forage and feces, it is recommended that samples should be freeze-dried for n-alkane analysis.

*In vitro* fermentation was used to determine if  $C_{31}$  was degraded in the rumen. Filter paper rated to retain particles greater than 25µm was used to isolate the residue because earlier work indicated n-alkanes are associated with the particulate phase of digesta. The amount of  $C_{31}$  found in residues collected by filtration after a 48-hour *in vitro* fermentation

**Table 1. Apparent dry matter digestibility.**

	<i>In vivo</i> DMD	$C_{31}$	$P <^a$	IADF	$P <^b$	<i>In vitro</i> DMD
----- % DM -----						
Meadow	67.5	62.9	.004	57.2	.0013	61.5
Range	70.5	61.8	.004	58.3	.14	58.3
Regrowth	70.7	57.5	.002	51.0	.06	57.8
Alfalfa	60.2	50.0	.01	43.8	.06	58.2
Meadow Hay	55.1	36.2	.0001	42.6	.01	47.1

<sup>a</sup> Comparison between *in vitro* digestibility and digestibility predicted using  $C_{31}$ .

<sup>b</sup> Comparison between digestibilities predicted using  $C_{31}$  and IADF.

period decreased ( $P < .001$ ) over 100 mg/kg. However, samples incubated for 96 hours produced residues similar ( $P = .78$ ) to those produced after 48 hours of incubation. Initial examination of the results indicated that  $C_{31}$  is highly degraded in the rumen. However, the digestibility trials with the same forages showed an average total tract recovery of 76.3 percent. Recovery of  $C_{31}$  in the residue left after *in vitro* fermentation was approximately 60 percentage units lower. While *in vitro* fermentation could degrade  $C_{31}$  to a greater extent than gastrointestinal passage, large differences are unlikely. Because the  $C_{31}$  amounts found in the residues remained unchanged between 48-hour and 96-hour incubation times, we propose low recovery was due to association of the marker with the liquid phase which was lost during filtration rather than degradation. Further examination is recommended to determine the digesta phase with which  $C_{31}$  associates during gastrointestinal passage.

Locating the site of n-alkane disappearance is important when evaluating its use as a potential internal marker. If disappearance is isolated to the lower tract, the marker may be used to estimate forage dry matter digestibility in the rumen. Because n-alkanes need to be intimately associated with the material they are marking to be reliable as internal markers, it is important the digesta phase association of n-alkanes be determined.

We concluded that: 1) in grazing situations where internal markers need to be used and dosing of synthetic n-alkanes is not practical, naturally occurring n-alkanes may be a better alternative to IADF for immature forages even though digestibility will still be underestimated; 2)  $C_{31}$  recovery was not consistent across forages; and 3) freeze-drying should be used to dry fecal samples for n-alkane analysis.

<sup>1</sup>Russell Sandberg, research technologist; Don Adams, professor, West Central Research and Extension Center, North Platte; Terry Klopfenstein, associate professor, Rick Grant, associate professor, Animal Science, Lincoln.

# Protein Evaluation of Treated Soybean Meal Products

Ryan Mass  
D. J. Jordon  
Terry Klopfenstein<sup>1</sup>

Treated soybean meal products vary in undegraded intake protein concentration and true nitrogen digestibility. Therefore, the value of these products in ruminant diets also varies.

## Summary

*Three treated soybean meal (SBM) products: 1) nonenzymatically browned SBM (Soy Pass®); 2) expeller SBM (SoyPlus®); and 3) a product of an unpublished manufacturing process (AminoPlus®), were compared using the following measurements: undegraded intake protein concentration (UIP), true nitrogen digestibility (TND) and metabolizable protein (MP) concentration. Soy Pass had the highest UIP, TND and MP values of the three treated SBM, followed by AminoPlus and then SoyPlus. The degree of heating may explain the differences in the three treated SBM products.*

## Introduction

Although soybean meal is the most commonly used protein supplement in the United States, the amount of metabolizable protein (MP) it supplies is not optimal because it is highly degradable in the rumen. The value of soybean meal for ruminants can be greatly enhanced by nonenzymatic browning (also known as the Maillard reaction). This chemical reaction complexes the protein with carbohydrate, increases its undegraded intake protein (UIP) concentration and increases MP supplied to the animal if intestinal digestibility is not reduced. However, excessive browning polymerizes the protein and decreases true nitrogen digestibility (TND).

Several commercial sources of soybean meal treated (TSBM) to increase UIP are available and each source is processed under different conditions. The objective of this research was to compare the UIP concentration, TND and MP supplied by each of three TSBM products.

## Procedure

In this digestion study, 15 crossbred wether lambs (70 lb) were utilized. All lambs were fed a common basal diet at 2.5 percent of body weight (DM basis; Table 1). The basal diet was formulated to contain a minimum of 10 percent CP, .42 percent Ca and .18 percent P. Urea was included to ensure rumen ammonia did not limit digestion and to provide 40 percent of the basal dietary nitrogen (N).

Three TSBM products were obtained for protein evaluation: 1) nonenzymatically browned TSBM (Soy Pass®); 2) TSBM (SoyPlus®); and 3) a TSBM product of an unpublished manufacturing process (AminoPlus®). Commodity soybean meal was also evaluated. Three lambs in each period were fed only the basal diet and served as a urea control. The remaining 12 lambs consumed the basal diet at the same percentage of body weight (DM basis) as control lambs, with an additional 3.75 percent of the basal diet DM added as units of N from one of the TSBM. Treatment diets were

(Continued on next page)

**Table 1. Composition of basal diet**

Item	Percent of diet DM
Cottonseed hulls	72.63
Dehydrated alfalfa pellets	15.00
Molasses	5.00
Dry-rolled corn	5.00
Urea	1.48
Dicalcium phosphate	.34
Sodium chloride	.30
Ammonium sulfate	.17
Sheep trace mineral premix	.04
Vitamin premix	.03
Selenium premix	.02